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Cell-free degradation assays

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An abbreviated version of this protocol was published in Journal of Experimental Botany in May 2019

Chalcone synthase is ubiquitinated and degraded via interactions with a RING-H2 protein in petals of Paeonia 'He Xie'

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Detailed protocol

0.5 g the petal samples were fully ground in liquid nitrogen. 1ml extraction buffer (25 mM Tris–MES, pH 7.5, 1 mM $MgCl_2$, 4 mM PMSF, 5 mM DTT) was added into samples, vortex and mix thoroughly, then incubate on ice for 30 minutes,shake and mix every ten minutes. After centrifugation at 4 °C, 12000 g for 15-20 min. Transfer the supernatant to a new tube, the total protein was in the supernatant. The extracted supernatant was used for degradation assays. The protein concentration was detected by Bio-Rad.

For recombinant His-PsCHS protein purification, full length of *PsCHS* gene was constructed in the pEASY-E1 expression vector, which was contained His tag protein. The constructed vector containing *PsCHS* gene was transferred into BL21 (DE3) *E. coli* expression strain. It was induced overnight at 16 °C after addition of 0.2 mM IPTG. After collecting the bacteria, breaking the cells with ultrasonic wave, cleaning the mixed protein, eluting the target protein. The protein concentration was detected again.

For recombinant His-PsCHS protein degradation. The purified PsCHS protein was divided into two equal parts. 100 ng His-PsCHS protein was incubated with the extracts (contain 500 µg petal total protein) in 100 µl with 1mM ATP and incubation for 0, 40, 80 and 120 min at 25 °C. The specific 26S proteasome inhibitor MG132 (MedChemExpress) was added into one (the final concentration was 30 µM, 40 µM is the optimum concentration). DMSO was added into the others as control. Samples were taken at different time intervals to measure PsCHS protein abundance by western blot analysis using an anti-His-tag monoclonal antibody from immunized mouse (1: 5000, CWBIO, Beijing, China).

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Shu, Q. and Wang, L. (2021). Cell-free degradation assays. Bio-protocol Preprint. bio-protocol.org/prep1268.
2. Gu, Z., Men, S., Zhu, J., Hao, Q., Tong, N., Liu, Z., Zhang, H., Shu, Q. and Wang, L.(2019). Chalcone synthase is ubiquitinated and degraded via interactions with a RING-H2 protein in petals of Paeonia 'He Xie'. Journal of Experimental Botany 70(18). DOI: 10.1093/jxb/erz245

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